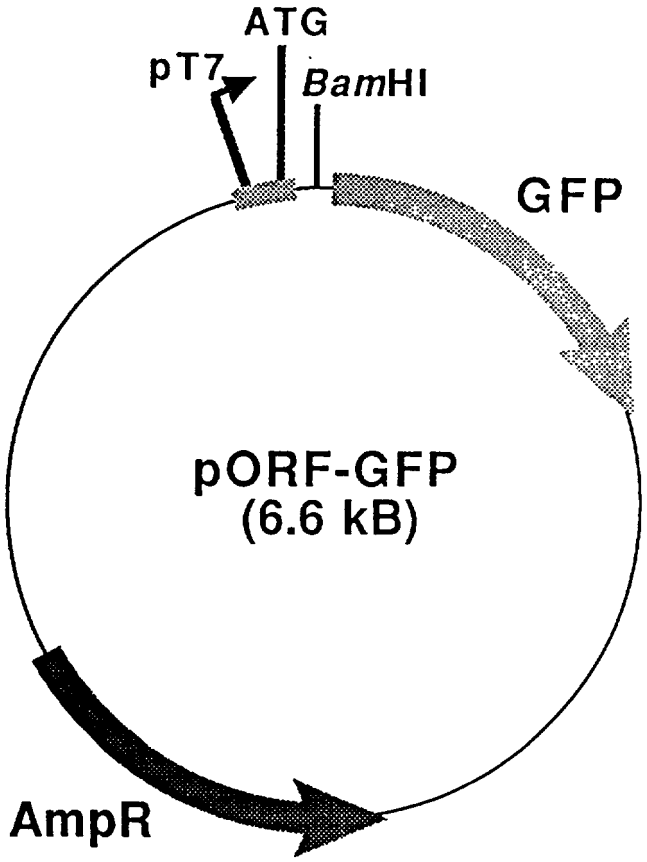

FIG. 1

GGT GCA GAT CTT GGA TCT CGT CCC GCG AAA

T7 Promoter

TTA ATA CGA CTC ACT ATA GGG AGA CCA CAA

CGG TTT CCC TCT AGA AAT AAT TTT GTT TCA

Shine-Dalgarno **Initiation codon**

CTT CAA GAA GGA GAT ATA CAT ATG **GGA TCC**

***BamH*I**

GGG CAG GTA AGT ATC AAG GTT ACA AGA CAA

GCT TAC ATA TG GFP

FIG. 2

GGT GCA GAT CTT GGA TCT CGT CCC GCG AAA

T7 Promoter

TTA ATA CGA CTC ACT ATA GGG AGA CCA CAA

CGG TTT CCC TCT AGA AAT AAT TTT GTT TCA

Shine-Dalgarno Initiation codon

CTT CAA GAA GGA GAT ATA CAT ATG GGA TCA

*Pac*I *BamH*I *Asc*I

TTA ATT AAC GGA TCC GGG CGC GCC GCT GCA

GCT CAA GCT TAC ATG CG GFP

FIG. 3

GGT GCA GAT CTT GGA TCT CGT CCC GCG AAA

T7 Promoter

TTA ATA CGA CTC ACT ATA GGG AGA CCA CAA

CGG TTT CCC TCT AGA AAT AAT TTT GTT TCA

Shine-Dalgarno **Initiation codon**

CTT CAA GAA GGA GAT ATA CAT ATG GGA TCA

*Pac*I *Nar*I *Asc*I

TTA ATT AAC GGC GCC GGG CGC GCC GCT GCA

GCT CAA GCT TAC ATG CG GFP

FIG. 4

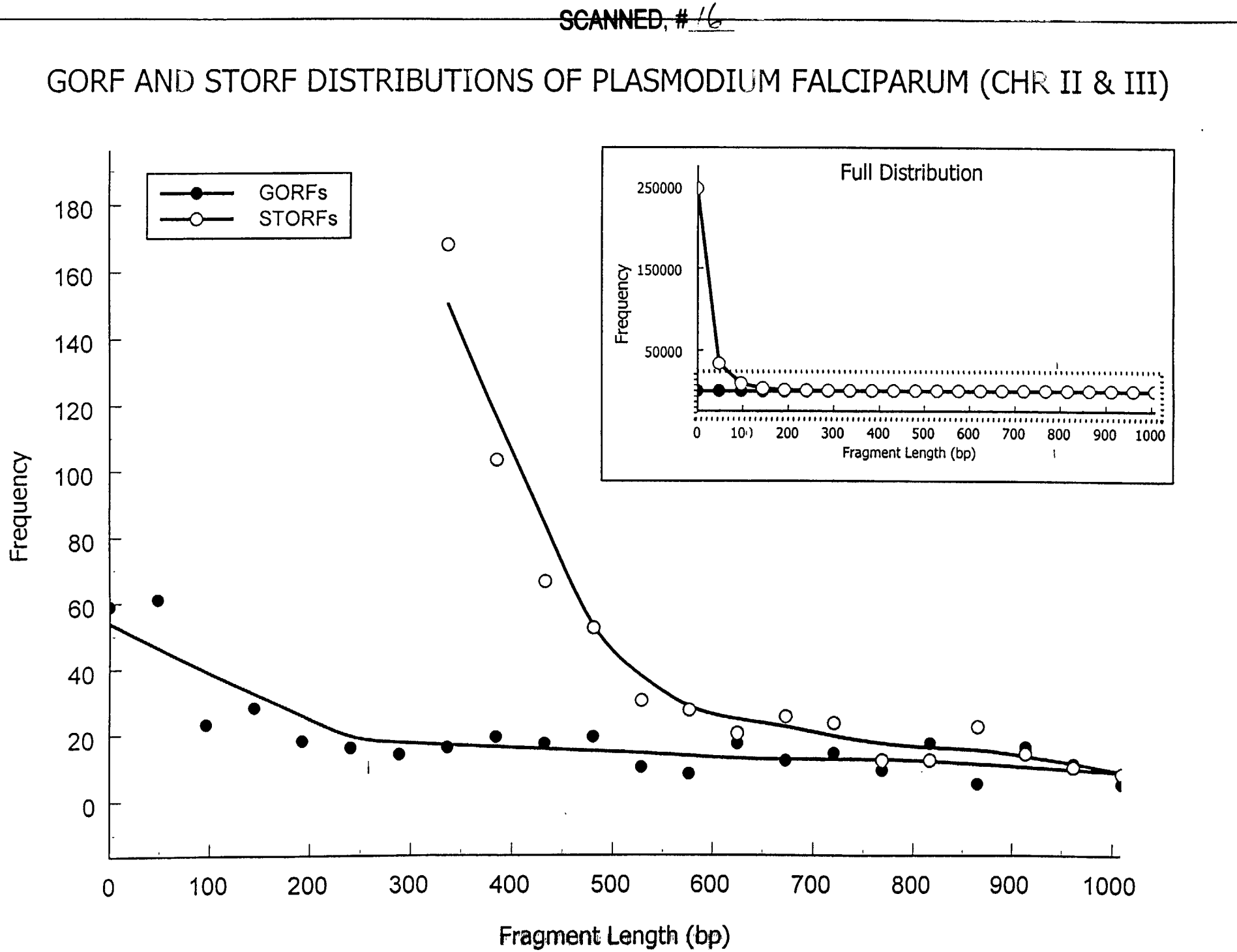

FIG. 5

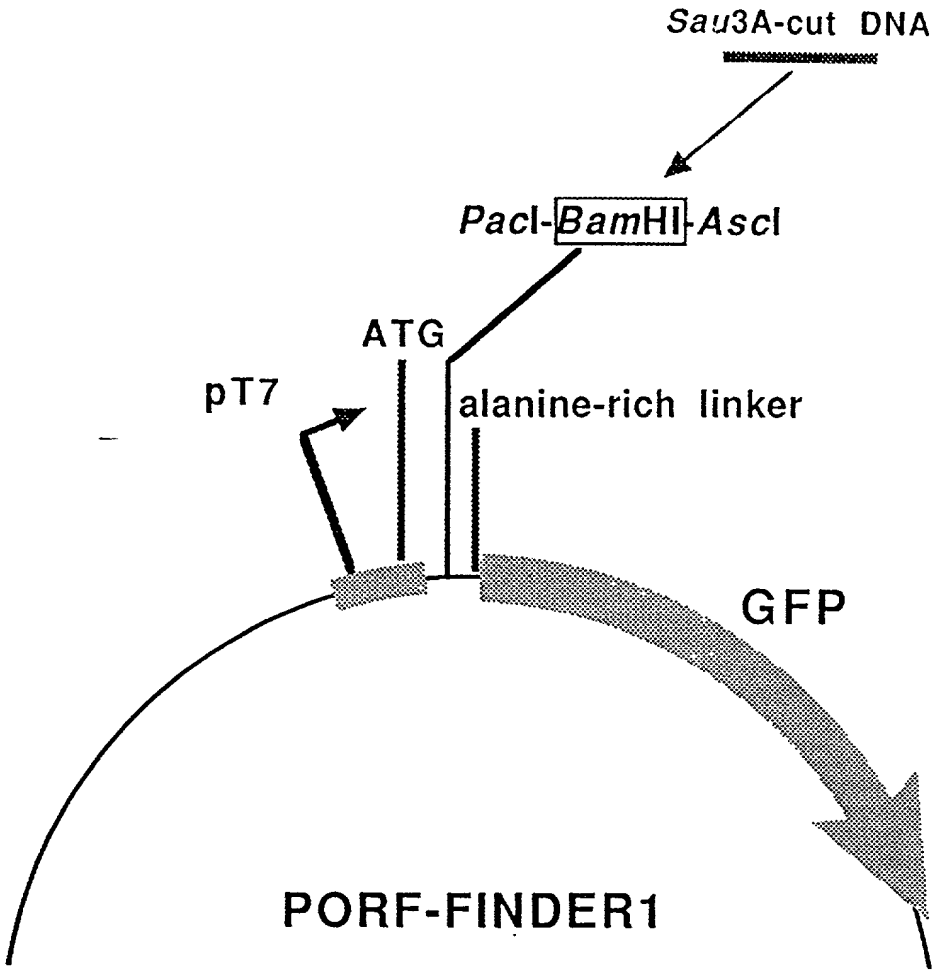
Sau3A-cut DNA
PacI-BamHI-AscI
ATG
pT7
alanine-rich linker
GFP
PORF-FINDER1

FIG. 6

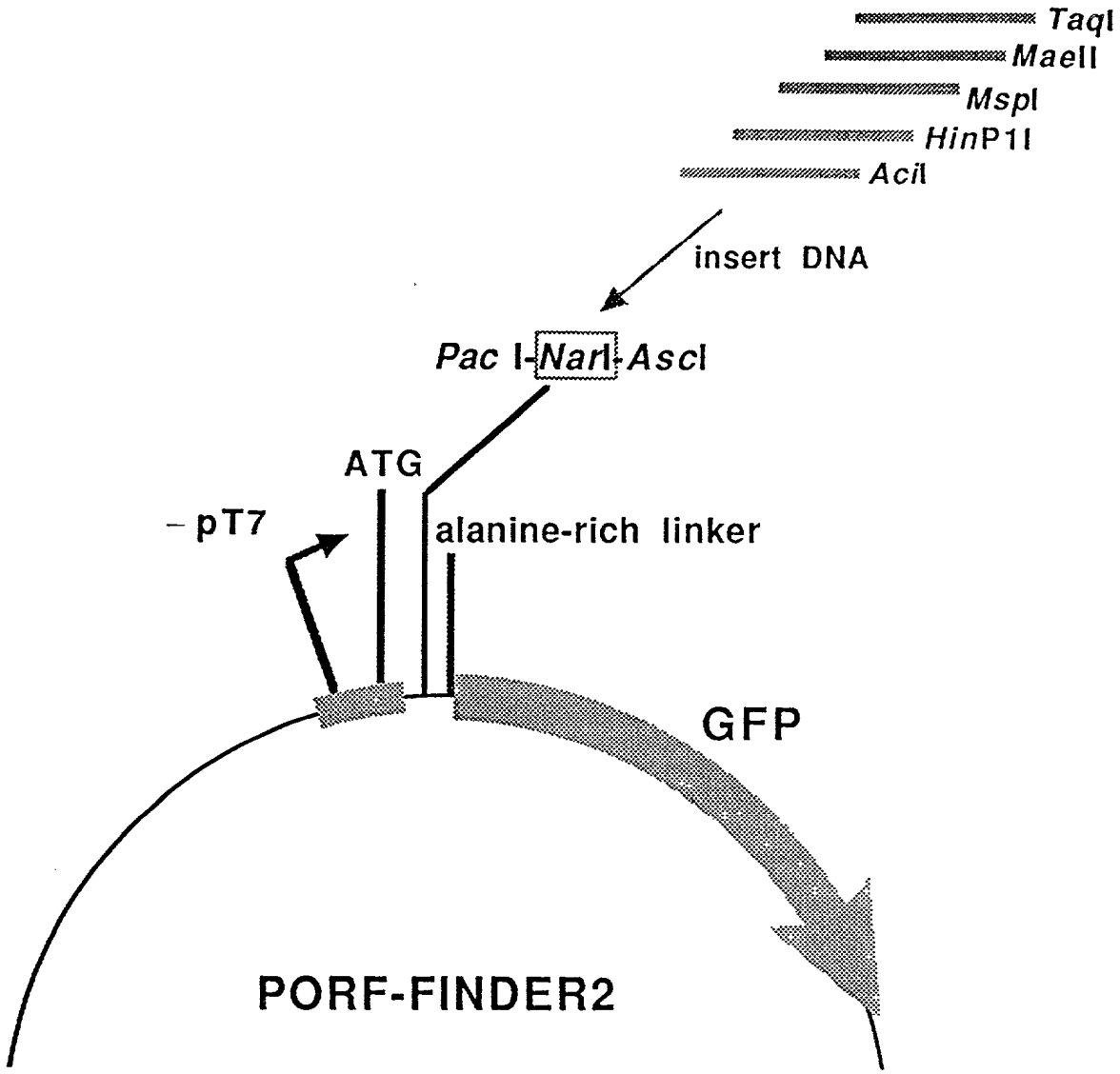
TaqI
MaeII
MspI
HinP1I
AciI
insert DNA
Pac I-NarI-AscI
ATG
pT7
alanine-rich linker
GFP
PORF-FINDER2

FIG. 7

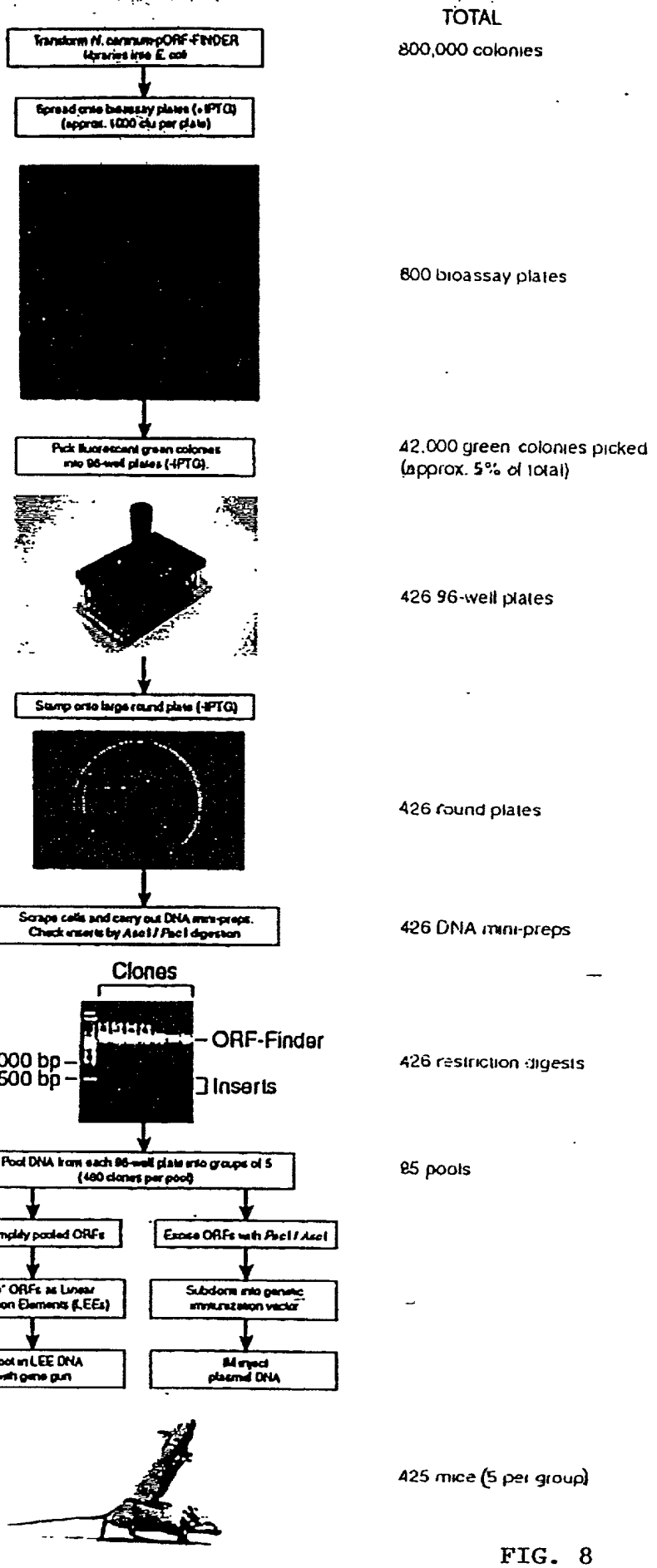
TOTAL
Transform N. carinum-pORF-FINDER libraries into E. coli
800,000 colonies
Spread onto bioassay plates (+IPTG) (approx. 1000 clu per plate)
800 bioassay plates
Pick fluorescent green colonies into 96-well plates (-IPTG).
42,000 green colonies picked (approx. 5% of total)
426 96-well plates
Stamp onto large round plate (-IPTG)
426 round plates
Scrape cells and carry out DNA mini-preps. Check inserts by AseI / PacI digestion
426 DNA mini-preps
Clones
ORF-Finder
1000 bp
500 bp
Inserts
426 restriction digests
Pool DNA from each 96-well plate into groups of 5 (480 clones per pool)
85 pools
PCR amplify pooled ORFs
Excise ORFs with PacI / AseI
"Clone" ORFs as Linear Expression Elements (LEEs)
Subclone into genetic immunization vector
Shoot in LEE DNA with gene gun
IM inject plasmid DNA
425 mice (5 per group)

FIG. 8